Survival of lichens and bacteria exposed to outer space conditions – Results of the *Lithopanspermia* experiments

Rosa de la Torre [a,*], Leopoldo G. Sancho [b], Gerda Horneck [c], Asunción de los Ríos [d], Jacek Wierzchos [d], Karen Olsson-Francis [e], Charles S. Cockell [e], Petra Rettberg [c], Thomas Berger [c], Jean-Pierre P. de Vera [i], Sieglinde Ott [f], Jesus Martinez Frías [g], Pablo Gonzalez Melendi [j], Maria Mercedes Lucas [d], Manuel Reina [a], Ana Pintado [b], René Demets [h]

[a] *INTA, Instituto Nacional de Técnica Aeroespacial, Crta. Ajalvir, km. 4, 28850-Torrejón de Ardoz, Madrid, Spain*
[b] *Universidad Complutense de Madrid, Departamento de Biología Vegetal II, Pza. Ramón y Cajal s/n, 28040 Madrid, Spain*
[c] *Deutsches Zentrum für Luft- und Raumfahrt, Institut für Luft- und Raumfahrtmedizin, Linder Höhe, 51170 Köln, Germany*
[d] *CSIC, Instituto de Recursos Naturales, Centro de Ciencias Medioambientales, Serrano 115dpdo, 28006 Madrid, Spain*
[e] *The Open University, Walton Hall, Milton Keynes, MK7 6AA, United Kingdom*
[f] *Heinrich-Heine-Universität, Institut für Botanik, Universitätsstr. 1, 40225 Düsseldorf, Germany*
[g] *INTA-CSIC, Centro de Astrobiología, Crta. Ajalvir, km. 4, 28850-Torrejón de Ardoz, Madrid, Spain*
[h] *ESA-ESTEC, Kepierlaan 1, 2201 AZ Noordwijk, The Netherlands*
[i] *Deutsches Zentrum für Luft- und Raumfahrt, Institut für Planetenforschung, Rutherfordstr. 2, 12489 Berlin, Germany*
[j] *Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, Campus de Montegancedo, Autopista M-38, Pozuelo de Alarcón, 28223 Madrid, Spain*

Keywords:
Astrobiology
Exobiology
Meteorites
Solar radiation

A B S T R A C T

In the space experiments *Lithopanspermia*, experimental support was provided to the likelihood of the lithopanspermia concept that considers a viable transport of microorganisms between the terrestrial planets by means of meteorites. The rock colonising lichens *Rhizocarpon geographicum* and *Xanthoria elegans*, the vagrant lichen *Aspicilia fruticulosa*, and endolithic and endoevaporitic communities of cyanobacteria and bacteria with their natural rock substrate were exposed to space for 10 days onboard the *Biopan* facility of the European Space Agency (ESA). *Biopan* was closed during launch and re-entry. In addition, in the *Stone* facility, one sample of *R. geographicum* on its natural granitic substrate was attached at the outer surface of the re-entry capsule close to the stagnation point, only protected by a thin cover of glass textolite. Post-flight analysis, which included determination of the photosynthetic activity, LIVE/DEAD staining, and germination capacity of the ascospores, demonstrated that all three lichen were quite resistant to outer space conditions, which include the full spectrum of solar extraterrestrial electromagnetic radiation or selected wavelength ranges. This high resistance of the lichens to space appears to be due to their symbiotic nature and protection by their upper pigmented layer, the cortex. In contrast, the rock- or halite-inhabiting bacteria were severely damaged by the same exposure. After atmospheric re-entry, the granite of the *Stone* sample was transformed into a glassy, nearly homogenous material, with several friction striae. None of the lichen cells survived this re-entry process. The data suggest that lichens are suitable candidates for testing the concept of lithopanspermia, because they are extremely resistant to the harsh environment of outer space. The more critical event is the atmospheric re-entry after being captured by a planet. Experiments simulating the re-entry process of a microbe-carrying meteoroid did not show any survivors.

1. Introduction

The concept of Panspermia, i.e. the propagation of microscopic forms of life between planets, originally formulated by Lord Kelvin (Thompson, 1871) and then further developed by Arrhenius (1903), was revived when meteorites were detected that originated from Mars (Becker and Pepin, 1984; Warren, 1994; Gladman, 1997). They provided evidence that rock fragments can travel from Mars to Earth in the solid state, and some of them experienced moderate shock pressures and temperatures (Melosh, 1984; Vickery and Melosh, 1987; Weiss et al., 2000; Head et al., 2002; Artemieva and Ivanov, 2004; Fritz et al., 2005). On Earth, a number of microbial communities have been found to inhabit subsurface environments (Pedersen, 2000). The rocks can act as a protective layer which protects the organism from environmental extremes, such as

desiccation (Friedmann, 1980; McKay and Friedmann, 1985), and high intensities of solar terrestrial radiation (Hughes and Lawley, 2003; Stivaletta and Barbieri, 2009). Recent studies have demonstrated the high tolerance of biological soil crusts to a wide variation in temperature (Büdel et al., 2009). In addition, some lichen species possess inherent protection mechanisms, e.g. their pigments are used as light filters (Solhaug et al., 2003; Gauslaa and Ustvedt, 2003; Gauslaa and Solhaug, 2004; Nybakken et al., 2004; Solhaug and Gauslaa, 2004; Stivaletta and Barbieri, 2009). Halite rocks in the Atacama Desert (Chile) harbour endolithic communities of microorganisms, such as *Chroococcidiopsis*-like cyanobacteria and associated heterotrophic bacteria (Wierzchos et al., 2006). It has been shown that a shallow layer of halite crystals protects those photosynthetic microbial communities from harmful UV radiation (Cockell and Raven, 2004; Fendrihan et al., 2009). Hypothetically, epilithic and cryptoendolithic microorganisms could be launched, with their host rock, from a planetary surface by an asteroid or comet impact (Mileikowsky et al., 2000; Clark, 2001) and therefore could act as an inoculum on a foreign planet. This concept, that life can be transferred from one planet to another by a rock is called "lithopanspermia" (Nicholson et al., 2000; Benardini et al., 2003; Cockell, 2008; Horneck et al., 2008). In this scenario, microorganisms have to cope with three major phases of stress: the escape from the home planet by impact ejection, a journey through space over extended time periods, and finally capture by another planet and atmospheric entry and landing.

Experimental evidence for a potential impact ejection of viable microorganisms from Mars and Mars-like planets has been provided in systematic shock recovery experiments simulating shock pressures between 5 and 50 GPa, a range that has been determined for martian meteorites (Artemieva and Ivanov, 2004; Fritz et al., 2005). This work demonstrated a well-defined launch shock window for the transfer of rock-inhabiting microorganisms from Mars to Earth by impact ejection, which ranged between 5 and 45 GPa for spores of *Bacillus subtilis* and the lichen *Xanthoria elegans*, but was restricted to 5–10 GPa for the rock-inhabiting cyanobacterium *Chroococcidiopsis* (Stöffler et al., 2007; Horneck et al., 2008; Cockell, 2008).

In space, microorganisms are confronted with an extremely hostile environment, characterised by a high vacuum (10^{-7}–10^{-4} Pa), an intense field of ionising radiation of solar and galactic origin, unfiltered solar UV radiation and extreme temperatures (−120 to +120 °C). Space technology has provided the facilities to study the survival of different microorganisms in the harsh environment of space (Demets et al., 2005; Baglioni et al., 2007). Among the organisms tested, spores of *B. subtilis* (Horneck et al., 1984, 2001; Horneck, 1993; Rettberg et al., 2002), the lichens *Rhizocarpon geographicum* and *X. elegans* (Sancho et al., 2007) and adults and eggs of the tardigrades *Richtersius coronifer* and *Milnesium tardigradum* (Jönsson et al., 2008) turned out to be the most resistant ones. *B. subtilis* (70%) spores survived 2107 days in space, on board of the NASA Long Duration Exposure Facility (LDEF), when shielded against solar UV (Horneck et al., 1994). However, direct exposure to the solar extraterrestrial UV radiation, reduced their survival by orders of magnitude. So far, lichens are the only organisms examined that were able to survive exposure to the complex matrix of all parameters of space including solar extraterrestrial UV radiation, as tested during the two-week flight of *Biopan*-5 (Sancho et al., 2007; de los Ríos et al., 2010). It has been suggested that this symbiotic system, composed of fungal and photosynthetic cells (cyanobacteria or algae), which is covered by a thick and pigmented cortex (Gauslaa and Solhaug, 2004), provides efficient shielding against the hostile parameters of outer space (Sancho et al., 2009).

If sufficiently shielded by meteorite-like material, endolithic microorganisms may survive the journey through space and finally be captured, with their host rock, by another planet. If the planet has an atmosphere, which might make it habitable (Lammer et al., 2009), the outer shell of the rocks is subjected to very high temperatures during atmospheric entry. So far, microorganisms that were embedded in 2 cm thick rocks fitted at the outer surface of a re-entry capsule (*Stone* facilities of *Foton* missions), thereby simulating the entry of a meteorite, did not survive this entry process (Brandstätter et al., 2008; Westall and de la Torre-Noetzel, 2008).

The aim of this work is to obtain further information on the mechanisms of the resistance of rock colonising microbial communities and lichens to outer space conditions, including space vacuum and different spectral ranges of solar extraterrestrial electromagnetic radiation. In the *Lithopanspermia/Biopan* experiment, a variety of microbial and symbiotic communities together with their natural habitat (rock or halite) were exposed to defined space conditions during the *Biopan*-6 flight of ESA on board of a Russian *Foton* satellite. Besides viability and ultra-structural integrity of the whole systems, we investigated the resistance of the ascospores to space and the role of the lichen cortex and pigments in protecting the underlying cells.

Complementary to *Lithopanspermia/Biopan* the aim of the *Lithopanspermia/Stone* experiment was to identify the physical, chemical and biological modifications caused by atmospheric entry in meteorites and to their possibly embedded microorganisms. We used the lichen *R. geographicum* as biological rock-inhabiting model system to test its ability to survive during entry into the Earth atmosphere.

With these studies we provided additional experimental evidence that microorganisms, residing in the interior of rocks, may be suitable candidates for an interplanetary transfer of microorganisms, as required by the lithopanspermia scenario.

2. Materials and methods

2.1. Spaceflight experiments

The experiment *Lithopanspermia/Biopan* was part of the payload of the *Biopan*-6 space mission of ESA mounted on the Earth-orbiting FOTON satellite. *Biopan* is a pan-shaped facility with the *Lithopanspermia/Biopan* hardware mounted within its lid (for further details see Appendix A). The biological samples were accommodated inside the *Lithopanspermia* hardware, which allowed access to space vacuum and selected wavelength ranges of solar extraterrestrial electromagnetic radiation: $\lambda > 110$ nm, $\lambda > 200$ nm, $\lambda > 290$ nm, $\lambda > 400$ nm, when in Earth orbit. *Biopan* was hermetically closed during launch and re-entry, and opened by telecommand after reaching its orbital altitude, thereby exposing the biological samples to the selected space parameters. Total flight duration was 10 days. After landing the samples were recovered and analysed.

The *Lithopanspermia/Stone* experiment was attached to the outer shell of the *Foton* re-entry capsule as part of the *Stone* facility. A rock sample with the lichen *R. geographicum* was directly mounted onto the outer shell of *Foton* with the biological layer facing the satellite. It was covered by a thin layer of a glass textolite, but otherwise it was open to space during launch, spaceflight and landing (see Appendix A for further details).

2.2. Biological samples

2.2.1. Lichens

Epilithic lichens were collected together with their rock substrate. The crustose lichen *R. geographicum* was collected at the Plataforma de Gredos (Sierra de Gredos, Avila, 2020 m a.s.l.,

40°17′N, 5°14′19″W), a region with a predominant lithology of granite with phenocrists, which is predominantly colonised by *R. geographicum* (Sancho et al., 2001). The area is characterised by a continental climate, where conditions (temperature variations, humidity, and solar UV and VIS radiation) are extremely harsh and where *R. geographicum* grows in "map-shapes", contributing to rock-weathering. Rocks with flat surfaces and homogeneous rich thalli colonisation (50 mm and more in diameter) were selected. In order to assess the protecting effect of the cortex, samples with intact cortex as well as those without cortex, which was mechanically removed by aid of a scalpel as described in de la Torre et al. (2007a) were used for the flight experiment. Although the latter method seems to be quite aggressive, field studies have shown that the photosynthetic activity of the lichen was not impaired by this treatment (de la Torre et al., 2007a).

Rocks with the epilithic placoid crustose lichen *X. elegans* (Link.) Th.Fr. were collected at Peñones de San Francisco (Sierra Nevada, Granada, Spain, 2400 m a.s.l., 37°06′N, 3°23′W). Samples with homogeneous covering of lichen thalli were selected. In order to assess the shielding effect of the pigments, samples with intact thallus as well as those without pigments, which were extracted by use of pure acetone 100%, according to Gauslaa and Ustvedt (2003), were used for the flight experiment.

Samples of *R. geographicum* and *X. elegans* with fruiting bodies (containing ascospores) were collected near the Sanetsch glacier and the Gornergrat glacier, Zermatt (Wallis, Switzerland, between 2000 m and 3300 m, GPS coordinates Sanetsch (2000 m): 46°21.799′N, 007°17.844′E, Zermatt/Gornergrat (3100 m): 45°59′ 06.68″N, 7°47′38.27″E, Zermatt/Hohthälli (3270 m): 45°59′21. 02″N, 7°48′09.86″E), in early summertime (mid of June) when the majority of the ascospores of *X. elegans* are fully developed in the mentioned habitat. For *R. geographicum*, the high degree of melanin incrustation and the anatomical and morphological structure of the ascospores in divided compartments were taken as indication of the final stage of spore development.

For the *Lithopanspermia/Biopan* experiment of each lichen/rock type, 12 cylindrical samples (9.5 mm in height, 6.9 mm in diameter, less than 1 g in weight) were cut by use of a diamond point saw machine (Fig. 1A). They were divided in three parallel sets of four samples serving as flight samples, flight spare samples and ground control samples, respectively. For *Lithopanspermia/Stone* a disc-shaped sample, with 45 mm diameter and 4 mm thickness, was cut out of a granite rock colonised with a homogeneous thallus of *R. geographicum* (Fig. A3A).

The vagrant lichen *Aspicilia fruticulosa* was collected on clayey soils in continental high basins of Central Spain (Guadalajara, Zaorejas, 40°45′14″N, 02°11′51″W, 1260 m a.s.l.). Vagrant or erratic lichens living unattached to the substrate are well known from the continental deserts and arid areas of Middle Asia, Eurasia, North America and Northern Africa. *A. fruticulosa* typically develops a globular fruticose and compact thallus up to 2.5 cm diameter, made up of numerous dichotomous or sympodial branching (Fig. 1B). At the top branches show more or less circular pseudocyphellae that appear as white regions which lack of cortical layer and expose the medulla to the atmosphere (Sancho et al., 2000).

2.2.2. Endoevaporitic microbial communities

Samples of halite (NaCl) crust with visible signs of colonisation in their cavities by endoevaporitic communities represented mainly by photoautotrophic cyanobacteria accompanied by heterotrophic bacteria as described previously by Wierzchos et al. (2006) were obtained from Salar Grande in the Atacama Desert

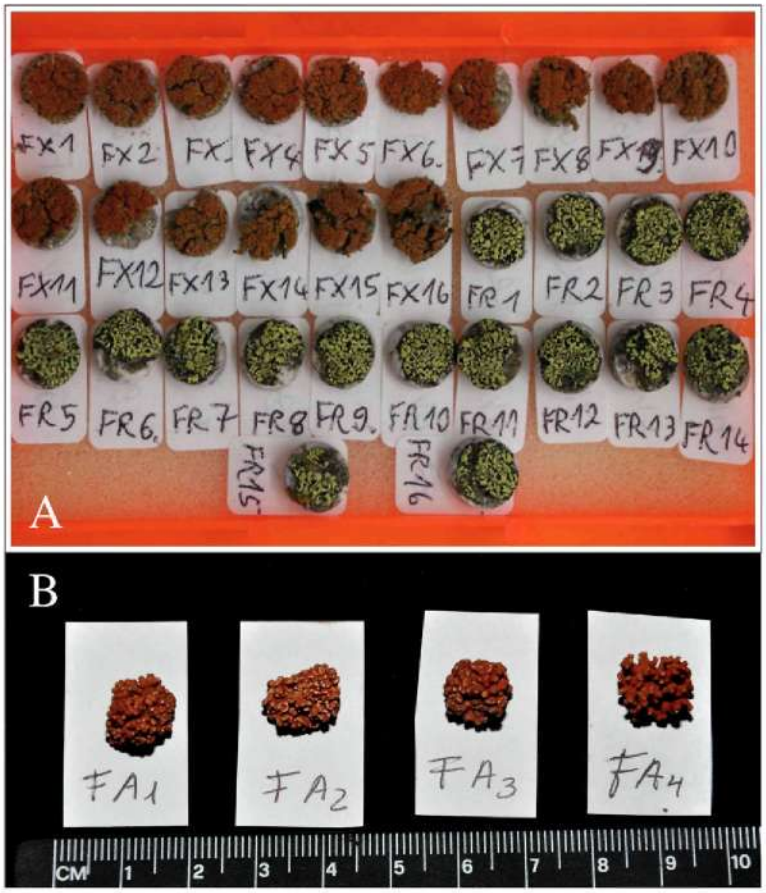

Fig. 1. Flight samples (A) of epilithic lichens on their natural rock substrate: *Xanthoria elegans* (FX) and *Rhizocarpon geographicum* (FR) and (B) of *Aspicilia fruticulosa*.

(20°55′30″S; 70°00′44″W). The samples for the *Lithopanspermia/Biopan* experiment were extracted in the form of small cores (9.5 mm in diameter and 6.9 mm high) from the colonised endolithic zone. This zone was distinguished by its light greenish colour arising from the presence of photoautotrophic microorganisms.

2.2.3. Endolithic cyanobacterial communities

Rocks naturally colonised by epilithic and boring (euendolithic) communities of cyanobacteria were sampled from coastal limestone/sandstone cliffs in Beer, Devon, UK. The rocks are colonised by a diverse cyanobacteria assemblage including species of *Leptolyngbya*, *Pleurocapsa* and *Phormidium* as determined from a 16S rDNA clone library and isolation studies on the communities (Olsson-Francis et al., 2010). The cliffs at Beer, are dominated by Cretaceous nodular chalk limestone, and the samples were collected from the upper greensand zone. The rock substrate itself is not as important as the microbial colonists which could potentially colonise any rock surface. However, sedimentary rocks such as the ones we examined have been shown to survive shock pressures associated with impact ejection and atmospheric entry (Brack et al., 2002; Horneck et al., 2008; Moeller et al., 2008). Samples of rock were cut into blocks with a surface of approximately 1 cm^2 for the *Lithopanspermia/Biopan* experiment.

To test the ability of resting states of cyanobacteria with known desiccation resistance to survive space conditions, the rocks were also seeded with akinetes (resting cells) induced from *Anabaena cylindrica* cultures (Yamamoto, 1975). *Anabaena* was obtained from the Pasteur Culture Collection (PCC 6309). The organism was grown in BG-11 medium (Rippka et al., 1979) at 25 °C, under natural sunlight and day/night cycle. Akinetes were induced by transferring log phase cells into an iron limited BG-11 medium, followed by three washes in the same medium. After five weeks of growth, the akinetes were harvested by allowing them to settle to the bottom of the flask. They were then washed in medium and transferred to dd H_2O. Akinetes were stored in a refrigerator at 4 °C until required. Akinetes (100 μl) were added to the surface of each of the rocks, and then dried. Samples were either used for the space experiment or as ground controls.

2.3. Rock samples

For the *Lithopanspermia/Stone* experiment a granite rock colonised with *R. geographicum* was collected at the Plataforma de Gredos (Sierra de Gredos, Avila, 2020 m a.s.l., 40°17′N, 5°14′19″W). Textural-, mineralogical- and geochemical analyses, as described in Section 2.5, were performed with two pieces of fresh granite previous to the space flight. The composition of the samples was: 50% feldspar (white to pink large subhedral prismatic crystals), 30% quartz (semi-transparent to white anhedral–subhedral crystals) and 20% mica (mainly elongated and fibrous-radial biotite and chlorite crystals). Quartz low (SiO_2), anorthoclase ((Na, K)$AlSi_3O_8$), albite, ordered ($NaAlSi_3O_8$), biotite ($K(Mg, Fe)_3AlSi_3O_{10}(F, OH)_2$) and ferroan clinochlore ($(Mg, Fe)_6(Si, Al)_4O_{10}(OH)_8$) were specifically detected by XRD. Feldspar grains showed incipient to advanced sericitic alteration and small fractures filled with quartz and micas. Irregular inclusions of ore minerals (mainly pyrite) occurred dispersed in the silicate groundmass (Fig. 2). Geochemically, the granite sample displayed significant amounts of Pb (21 ppm), Cr (25 ppm), Zn (81 ppm), V (85 ppm) and Zr (206 ppm).

2.4. Pre- and post-flight analysis of biological samples

2.4.1. Lichens

To determine the activity of the photosystem II (PSII) of the photobiont of the lichens, the samples of *R. geographicum*, *X. elegans* and *A. fruticulosa* were reactivated in a climatic chamber under

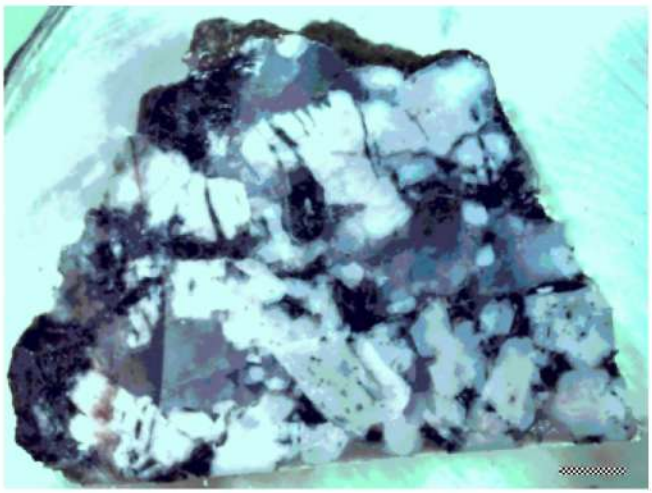

Fig. 2. Granite sample used in the *Lithopanspermia/Stone* experiment; polished section showing the types and morphologies of crystals and their textural relationships. Scale bar: 0.5 cm.

controlled conditions as follows: constant temperature of 10 °C, 12 h light and 12 h dark cycles for 72 h. Irradiation with photosynthetic active light was performed by use of a mercury lamp with a 100 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). For rehydration, the samples were sprayed twice a day with deionised water. Then the activity of the photosystem II (PSII) of the photobiont was measured by use of a Mini-PAM fluorometer (Heinz Walz GmbH), as described previously (Sancho et al., 2007; de la Torre Noetzel et al., 2007b). The lichens were rewetted immediately before each measurement. The optimum quantum yield of chlorophyll *a* was determined by fluorescence measurements after 20 min of dark adaptation according to Schreiber et al. (1994) from the equation:

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

with F_v = variable fluorescence yield, F_m = maximal fluorescence yield, and F_o = minimal fluorescence yield. This optimum quantum yield of photosystem II was taken as an indication of the PSII activity of the photobiont of the lichen system after the exposure to the space parameters. The relative PSII activity was determined from the ratio of the F_v/F_m of the flight sample to the pre-flight data of the same sample.

In addition, the viability of the lichens *R. geographicum* and *X. elegans* – of the entire lichen thallus, and separately of the mycobiont hyphae and photobiont cells embedded in the lichen symbiontic structure – was determined by Confocal Laser Scanning Microscopy (CLSM) (Leica TCS-NT/Confocal Systems Software) analysis of samples stained with LIVE/DEAD-dye, FUN I (INVITROGEN, Molecular Probes) (de Vera, 2005; de Vera et al., 2003, 2004a,b, 2008; Horneck et al., 2008; Onofri et al., 2008). For this LIVE/DEAD analysis a short reactivation time of 15 h was applied. Channel-imaging was correlated with contour images and overlay images to improve recognition of visual damage. Channel-imaging emission filters in three different fluorescence ranges were applied. Band pass filters at 548 nm, 559 nm, and 506 nm were used for green, red, and blue fluorescence, respectively. From the quantitative data on the viability of the exposed samples that were stained by LIVE/DEAD-dye, FUN I mean values were determined, which indicated the deviation of the ratio of vital tissue t_v to the whole lichen thallus t_o. Viable and non-viable cells of both symbionts, the photobiont and mycobiont, in the lichen tissue were quantified by the cell counting program of Image-J and by CLSM fluorescence

intensity diagram analysis. The relative viability was determined from the ratio of the viability of the flight sample to that of the ground control, kept at ESTEC (ambient temperature, dark) during the mission period. This LIVE/DEAD analysis is a direct indicator of the physiological activity of the sample.

For germination and growth capacity tests, fruiting bodies with ascospores of *X. elegans* and *R. geographicum* were washed with MILLIPORE filtered clean water to remove possible contaminations by bacteria or other fungal spores that may have occurred after opening of the *Biopan* hardware. After the washing procedure the samples were glued by use of neutral plastiline dots on the lids of Petri-dishes; the lids were then placed over the dishes, which were filled with a Malt Yeast/ampicillin (MY/amp) extract. A consecutive drying procedure caused the ascospores to leave the fruiting bodies and to spread on the MY/amp medium. Light microscope observations and photographic documentation were performed during the following weeks to determine the germination and growth capacity of space-exposed samples in relation to the ground controls. From the images, the rate of germination and growth (in percent) of space exposed ascospores was determined by use of the cell counting program Image-Tool.

Lichen thalli of *A. fruticulosa* were examined using the Low Temperature Scanning Electron Microscopy (LTSEM) technique following de los Ríos et al. (2005). Small lichen fragments were fixed onto the specimen holder of the cryo-transfer system (Oxford CT1500), plunged into sub-cooled liquid nitrogen, and then transferred to the scanning electron microscope (SEM) via an air-lock transfer device. The frozen specimens were cryo-fractured in the preparation unit and transferred directly via a second air lock to the microscope cold stage where they were etched for 2 min at −90 °C. After ice sublimation, the etched surfaces were gold-sputter coated in the preparation unit. Samples were subsequently transferred onto the cold stage of the SEM chamber. Fractured and etched surfaces were observed under a DSM960 Zeiss SEM microscope at −135 °C under conditions of 15 kV acceleration potential, 10 mm working distance and a 5–10 nA probe current.

2.4.2. Endoevaporitic microorganisms

After space flight, the viability of endoevaporitic halite microbial communities, comprising photosynthetic and heterotrophic microbial communities, was tested using the nucleic acid double-staining (NADS) procedure, which combines a general nucleic acid dye – Sybr Green 1 (SB1) with a membrane integrity probe propidium iodide (PI) (Falcioni et al., 2008). Both dyes stain RNA and DNA (Haugland, 2002) yet differ in their spectral characteristics and their ability to penetrate healthy bacterial cells (Jones and Senf, 1985). When used together, propidium iodide only penetrates bacteria with damaged membranes, blocking SB1 green fluorescence. Thus, bacteria with intact cell membranes stain fluorescent green, while bacteria with damaged membranes appear fluorescent red. This bacterial viability kit is a sensitive, single-step assay for discriminating between live and dead bacterial cells. NADS analysis was conducted on powdered core samples by introducing 1/3 of each sample weighing around 0.2 mg in Eppendorf tubes and dissolving in 1 ml of sterile water. After 5 min of sedimentation, the supernatant suspension containing the cells was placed in another Eppendorf tube and after centrifugation, the pellets were stained according to the NADS protocol (Falcioni et al., 2008). Each pellet was stained for 10 min with 10 μl of SB1 solution (final dilution, 1:1000 [vol/vol]) followed by the addition of 10 μl of PI (PI final concentration 10 μg/ml). All stained samples were observed using a Zeiss Axio Observer A1 fluorescence microscope. A CCD Zeiss camera and Carl Zeiss Axio Vision software were used to capture and record the green (SB1) and red (PI) signals.

2.4.3. Endolithic cyanobacteria

To test for viable endolithic cyanobacteria after space flight, the rocks with endolithic microbial communities were halved and incubated in 5 ml of BG-11 medium and filtered sea water. The samples were exposed to sunlight and natural day/night cycles at 25 °C, for two months. The surfaces of the rocks were scraped with a blade and inoculated into BG-11 or seawater media and spread onto plates of the same composition. Cyanobacteria were identified by morphological and molecular techniques as follows: (i) examination at 1000 times magnification on a Leica DMRP microscope equipped with a epifluorescence microscope, which allowed to determine whether the cyanobacteria were in resting or vegetative state; (ii) identification of cyanobacteria by amplification of the 16S rDNA gene by PCR and specific primers (Nubel et al., 1997). The reaction mixture contained: 5 μl of culture; 200 μM dNTP; 1 μM primers; 5 U of *Taq* DNA polymerase; 1 × PCR Buffer (20 mM Tris–HCl (pH 8.4), 50 mM KCl) and 2.5 mM MgCl (Invitrogen, Paisley, UK). Amplification consisted of incubation at 94 °C for 15 min: this was followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and a 1 min extension at 72 °C; with a final extension of 5 min at 72 °C. Sequences were phylogenetically classified and their nearest 16S rDNA sequences identified in the GenBank database.

2.5. Pre- and post-flight analysis of rock samples of Lithopanspermia/Stone

Analyses of the textural, mineralogical and geochemical characteristics of the granitic rocks of *Lithopanspermia/Stone* experiment were performed using the following set of analysing techniques: a combination of transmitted and reflected light microscopy (Nikon E600 POL polarising microscope), X-ray Diffraction (Seifert XRD 3003 T-T), Fourier transform infrared spectroscopy (Nexus Nicolet FTIR) with a microscope attached (Spectra-Tech IR-Plan Microscope) and inductively coupled plasma mass spectroscopy (ELAN 9000 ICP-MS).

3. Results

3.1. Lithopanspermia/Biopan

Within the *Lithopanspermia/Biopan* experiment the biological samples were exposed to the different parameters of space according to the sample-test-parameter plan (Table 1). After retrieval, the samples were distributed to the different laboratories for analysis of the effects of the space parameters applied to them.

3.1.1. Lichens after space exposure

The PSII activity of the flight samples of the lichens *R. geographicum*, *X. elegans* and *A. fruticulosa* was determined after 72 h of hydration and pre-adaptation of the desiccated specimens (Table 1). Intact thalli of all three lichen species that were exposed to space environment except solar electromagnetic radiation (dark flight controls) reached 100% PSII activity within experimental error compared to the pre-flight data of the same samples. These data show that the photosynthetic activity of the samples had rapidly recovered after the flight. A similar high relative PSII activity was measured in the ground laboratory controls (data not shown). Irradiation with solar extraterrestrial radiation during the space mission did not significantly reduce the PSII activity of the lichens, irrespective of the wavelength range applied (Table 1). Only in *X. elegans*, exposed to >400 nm sunlight, the PSII activity was reduced by 20%. This extremely high resistance of the photosynthetic systems of the lichens to outer space conditions, including solar extraterrestrial electromagnetic radiation, confirms earlier observations

Table 1
Biological test systems of the *Lithopanspermia/Biopan* experiment, the space parameters tested and their effects on the biological systems; all samples were exposed to space vacuum, cosmic radiation and selected wavelength ranges of solar extraterrestrial electromagnetic radiation (UV/VIS).

Biological system[a]	Specification	Space parameter		Relative PSII activity (%)	Viability (LIVE/DEAD analysis) (%)	Germination capacity (%)
		Cosmic radiation (mGy)	UV/VIS (nm)			
Rhizocarpon geographicum	Intact thallus	4 ± 0.5	Dark	93.8 ± 11.4	78.7 ± 8.8	93.38 ± 4.7
		100 ± 20	>110	94.5	52.3 ± 9.9	81.0 ± 28.7
		100 ± 20	>200	102.6	77.9 ± 9.2	87.9 ± 13
		100 ± 20	>290	99.7	63.5 ± 9.1	97.9 ± 3.2
		100 ± 20	>400	115.1	78.5 ± 10.3	93.8 ± 13.8
	Cortex removed	4 ± 0.5	Dark	95.9 ± 13.4	ND	NA
		100 ± 20	>110	77.1	ND	NA
		100 ± 20	>200	95.7	ND	NA
		100 ± 20	>290	87.2	ND	NA
		100 ± 20	>400	34.1	ND	NA
Xanthoria elegans	Intact thalli	4 ± 0.5	Dark	100.3 ± 4.0	82.4 ± 6.2	91.12 ± 8.6
		100 ± 20	>110	91.4	69.6 ± 5.7	75 ± 20.2
		100 ± 20	>200	98.0	75.5 ± 13.7	82.8 ± 11.7
		100 ± 20	>290	95.3	72.9 ± 13.6	82.5 ± 16.7
		100 ± 20	>400	80.6	67.2 ± 9.2	87.5 ± 14.1
	Depigmented	4 ± 0.5	Dark	76.6 ± 19.6	ND	NA
		100 ± 20	>110	89.8	ND	NA
		100 ± 20	>200	55.2	ND	NA
		100 ± 20	>290	95.2	ND	NA
		100 ± 20	>400	55.7	ND	NA
Aspicilia fruticulosa		4 ± 0.5	Dark	100.0 ± 1.8	ND	NA
		100 ± 20	>110	99.5	ND	NA
		100 ± 20	>200	100.0	ND	NA
		100 ± 20	>290	95.5	ND	NA
		100 ± 20	>400	96.6	ND	NA

NA = not applicable.
ND = not determined.
[a] In addition, samples of endoevaporites and endolithic cyanobacteria were exposed to the same conditions.

made in the experiment LICHENS on board of *Biopan*-5 (Sancho et al., 2007, 2009).

In order to test whether the cortex with its pigments served as protective Sun-screen (Solhaug et al., 2003; Gauslaa and Solhaug, 2004), a set of samples of *R. geographicum*, of which the cortex had been removed before flight, was exposed to the same space conditions. Compared to the pre-flight data, their PSII activity was not affected in the dark flight samples, however most Sun-exposed samples ($\lambda > 110$ nm, $\lambda > 290$ nm and $\lambda > 400$ nm) showed a reduced, relative PSII activity (Table 1). Considering the results of all Sun-exposed cortex-depleted samples together – regardless of the wavelength range applied, we observe a high variation between the PSII activity of the individual Sun-exposed samples. The reason for this variation of "cortex-depleted" lichens may be explained by a possible incomplete removal of the cortex in some samples, that show high PSII activity, e.g. those exposed to $\lambda > 200$ nm and $\lambda > 290$ nm. Surprisingly, flight samples exposed to solar radiation of $\lambda > 400$ nm showed the highest reduction in PSII activity. Unfortunately, the limited space in the *Lithopanspermia* flight hardware did not allow accommodating more than one sample per test parameter, so that a statistical analysis of the data is not possible. When comparing the data of the Sun exposed to the flight dark samples, they support the suggestion that the cortex with its pigments acts as a protective endogenous shield against solar electromagnetic radiation. Sun-screening pigments are well known from different lichen species (Solhaug et al., 2003), especially in those living in Arctic habitats (Nybakken et al., 2004) and in high mountain areas, where the cortex protected *R. geographicum* from the harsh environment governing that milieu (de la Torre et al., 2007a). A Sun-screening effect of the pigments was also observed in flight samples of *X. elegans*, where a parallel set of samples had been depigmented before flight (Table 1). Their relative PSII activity was reduced, at least in samples exposed to solar extraterrestrial radiation in the ranges of $\lambda > 200$ nm and $\lambda > 400$ nm. However, because the PSII activity of the depigmented dark flight samples was (76.6 ± 19.6%) lower than that of the intact dark flight samples (100.3 ± 4.0%), it is more likely that depigmentation per se made the lichens more sensitive to the complex matrix of all parameters of space applied to them. Again, more studies in space with more samples are required in order to assess the significance of those observations.

Viability of the lichens *R. geographicum* and *X. elegans* after space exposure was determined as active staining index, i.e., the rate of viable to non-viable cells of the lichen tissue measured by the CLSM technique. In this case, the viability index of the flown dark controls of both species (ranging from about 79% to 82%) was slightly reduced compared to the ground control (94%) (Table 1). The Sun-exposed flight samples showed a higher loss in viability than the dark controls. *R. geographicum* was especially damaged by the full spectrum of solar extraterrestrial radiation ($\lambda > 110$ nm), when the viability dropped to 52.3% (compared to 92% viability of the ground controls). Viability of Sun-exposed flight samples of *X. elegans* was reduced to values from 67% to 75% (compared to ground control of 95%), however a dependence of viability on the spectral range of solar UV was not observed (Table 1).

The CLSM technique allows also differentiating between damaged photobiont and mycobiont cells. Whereas in *X. elegans* photobiont cells were more affected (less stained by FUN 1 and no physiologic activity), in *R. geographicum* a higher degree of damage occurred in the mycobiont cells. Because in both investigated species, 2/3 of the lichen thallus is composed of mycobiont cells, the higher sensitivity of the mycobiont cells in *R. geographicum* is the reason for its higher loss of viability compared to *X. elegans* (Table 1).

Ultrastructural analysis by LTSEM technique revealed the integrity of both the algal (black arrow) and fungal (white arrow) cell walls in thalli of *A. fruticulosa* exposed to the full spectrum of solar extraterrestrial radiation of $\lambda > 110$ nm, (Fig. 3).

3.1.2. Germination and growth capacity of ascospores after space exposure

After space exposure, ascospores were isolated from the thalli of *R. geographicum* and *X. elegans* and their germination and growth were studied. For all flight samples, the ascospores of *X. elegans* showed a germination index between 75% and 90% (Table 1) (compared to 97% of the ground control). The highest loss in germination was found in ascospores from samples exposed to the full extraterrestrial solar UV ($\lambda > 100$ nm). An even higher germination index (81–100%) was found for *R. geographicum* flight samples.

After 1–3 days of incubation, the ascospores of *X. elegans* flight samples started germination by forming the initiation hypha. This was 1–2 days earlier than observed for the ground controls (starting point 2–4 days). The following steps were identical with the controls: first branching after 10 days and the formation of network mycelia after 22 days.

In contrast to the flight samples, ascospores of the ground controls of *R. geographicum* did not show any germination under the applied laboratory conditions (0% germination index). It seems that the space conditions have triggered the germination process in the ascospores of *R. geographicum*, so that they started germination, when brought in contact with the nutrient medium in the laboratory. In their natural habitats in high mountain regions (2500–3200 m), desiccation is essential for breaking up the protective ascosporic membranes and cell walls to allow germination and growth of the lichen mycobiont of *R. geographicum*. Probably, desiccation by space vacuum exerted a similar triggering of germination in the ascospores of the flight samples of *R. geographicum* as it occurs in nature.

3.1.3. Endolithic halite microbial communities after space exposure

The applied NADS technology enables differentiation between bacteria with intact and damaged cytoplasmic membranes (Berney et al., 2007), which to a certain extent allows also differentiating between active and dead cells. In Fig. 4 the live bacterial cells show

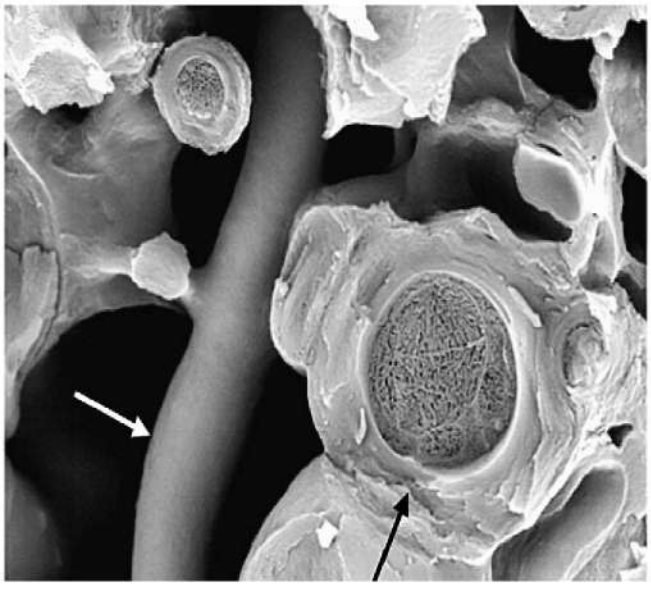

Fig. 3. LTSEM observation of the thallus of *Aspicilia fruticulosa* flight samples exposed to UV of >110 nm. Black arrow indicates algal cells and white arrow fungal cell.

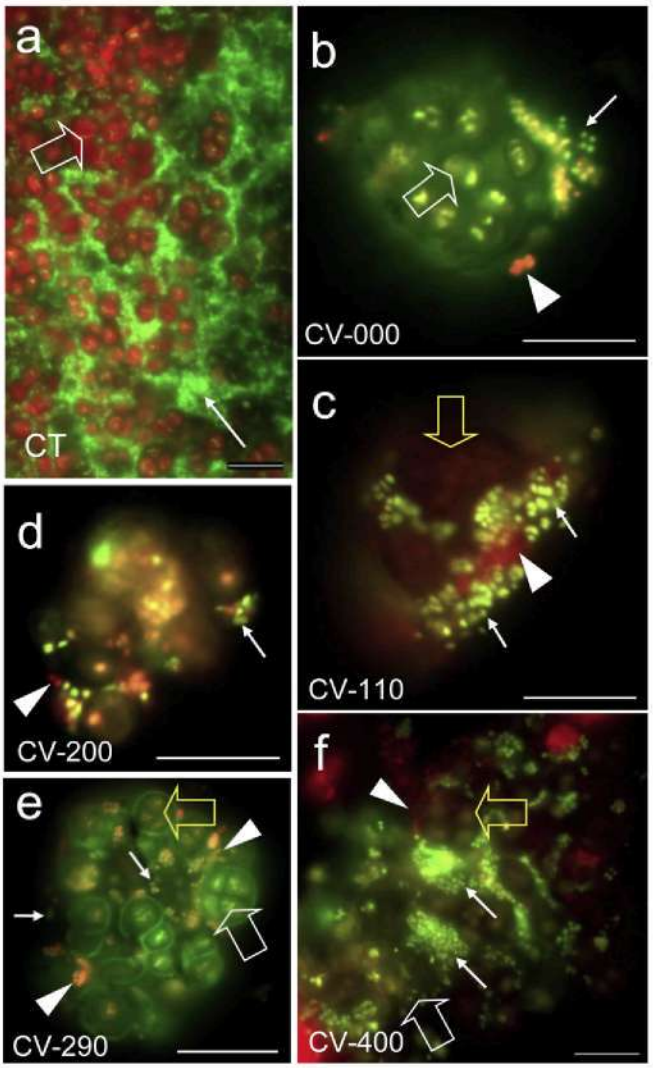

Fig. 4. Fluorescence microscopy images of endoevaporitic cyanobacteria and heterotrophic bacteria after the space flight within their halite rock substrate. All samples were stained with the nucleic acid fluorescent assay reagents Sybr Green I (SB1) and propidium iodide (PI); scale bars = 20 μm. (a) Ground control sample (CT) showing live cyanobacteria (open arrow) and heterotrophic bacterial cells (arrow); (b) dark flight sample revealing live cyanobacteria (open arrow), and live (arrow) and dead (arrowhead) heterotrophic bacteria; (c) flight sample exposed to UV at >110 nm, showing dead microorganisms (cyanobacteria, yellow open arrow; bacteria, arrowhead) and live (arrows) bacterial cells; (d) flight sample exposed to UV at >200 nm showing live (arrow) and dead (arrowhead) bacterial cells; (e) flight sample exposed to UV at >290 nm showing live cyanobacterial cells (white open arrow), dead cyanobacteria cells (yellow open arrow), and live (arrows) and dead (arrowhead) heterotrophic bacteria; (f) flight sample exposed to VIS (>400 nm) showing live (arrows) and dead (arrowhead) heterotrophic bacteria, as well as live (white open arrow) and dead cyanobacteria (yellow open arrow) cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

green fluorescence and dead bacteria emit a red fluorescence signal. Fig. 4a gives an example of live cyanobacteria and live heterotrophic bacteria cells of the ground control. Note the intense red autofluorescence of the photoautotrophic cells (open arrow in Fig. 4a). Nucleic acids stained with SB1, appeared as yellow spots (green SB1 signal on a background of red autofluorescence gives a net yellow signal) within the cyanobacterial cytoplasm, indicating cell membrane integrity and thus their viability. Live heterotrophic bacterial cells were also distinctly stained with SB1 (arrow in Fig. 4a). Fig. 4b shows microorganisms of a flight dark sample. This image reveals a high proportion of live cyanobacterial and

heterotrophic bacterial cells (green SB1 signals). In the flight sample that was exposed to the full spectrum of solar extraterrestrial electromagnetic radiation (>110 nm), cell integrity was less preserved (Fig. 4c). Most of the cyanobacteria and heterotrophic bacteria cells in this sample showed red PI fluorescence indicating dead cells with damaged membranes. Only around a tenth (visual estimate and counting) of the heterotrophic and photoautotrophic cells emitted green fluorescence indicating they were intact and alive. In addition, one has to consider, that – whereas the membrane-compromised bacterial cells can be clearly considered dead – those cells that appear "intact" may also be damaged to a certain extent. This has especially been observed for UV-irradiated bacteria (Villarino et al., 2000). Comparable images were obtained for flight samples that were exposed to solar extraterrestrial radiation at $\lambda > 200$ nm (Fig. 4d). The percentage of live cyanobacteria cells as well as heterotrophic bacteria gradually increased from flight samples exposed to solar extraterrestrial radiation at $\lambda > 290$ nm (Fig. 4e) to those, exposed solely to VIS ($\lambda > 400$ nm). In the latter case, the quantity of live heterotrophic bacterial cells was comparable to that observed in dark flight samples.

3.1.4. Cryptoendolithic microbial communities from the Beer rock after space exposure

Addition of BG-11 and seawater to the rocks from the ground control experiment resulted in green mats forming on the surface of the rocks after eight weeks. The green mats were cultured, and after one month, growth was visible on the plates (Fig. 5A). Examination of the colonies under the microscope identified both vegetative and resting state cyanobacteria. The predominant vegetative morphology was identified by 16S rDNA analysis as *Leptolyngbya* species. *Leptolyngbya* are prevalent in the 16S rDNA library prepared from the natural rocks (Olsson-Francis et al., 2010).

For rocks exposed to Low Earth Orbit (LEO) conditions in the *Lithopanspermia* experiment, the green epilithic growth on the surface of the rock turned brown after two days (this was not observed with the ground control rocks). After eight weeks, no green mats were distinguishable; however, the surface was spread onto plates to test for growth. After a month, green colonies appeared on the rocks that were not exposed to the Sun, i.e. dark control flight samples. The green filamentous colonies grew from small fragments of rock on the plate (Fig. 5B and C). Microscopic studies revealed that colonies from the space-exposed samples were the same as those of the ground controls. The organisms grew as long chains of spherical cells morphologically identical to *Anabaena* (Fig. 5D). 16S rDNA PCR using cyanobacteria specific primers confirmed them to be *Anabaena*.

3.2. Results of Lithopanspermia/Stone

3.2.1. Mineralogical, textural and geochemical changes of the granite during the atmospheric re-entry process

The *Foton* capsule with the *Stone* facility entered Earth's atmosphere with a velocity of 7.7 km/s, a velocity, which was below the 12–20 km/s of medium sized meteoroids. The atmospheric re-entry process of the granite sample in the *Lithopanspermia/Stone* experiment caused a general mineralogical and geochemical transformation into a glassy, nearly homogeneous material (Fig. 6). At micrometric scale, it occasionally displayed imprints of atmospheric flight that resembled those found on meteorite fusion crust (e.g. friction striae), as well as some particular features (principally semi-transparent to black glass droplets and rough and clean areas), which are similar to those found in some meteorites and pseudometeorites (Genge and Grady, 1998; Martinez-Frias et al., 1999). SEM-EDX analysis of the glass indicated chemical variations (wt.%) of Na (0.77–1.96), Mg (0.40–2.89), Al (2.51–7.94), Ca (0.87–4.49), K (0.27–2.70), Si (14.45–24.25) and O (61.94–69.95). Some microdomains were made of pure silica.

3.2.2. Survival of the lichen R. geographicum after the atmospheric re-entry process

The lichen *R. geographicum* on its natural granite habitat was one of the four *Stone*-samples facility on *Foton* M3 facing to the backside. During the entry process the samples experienced temperatures that were high enough to melt silica and basalt. None of the biological *Stone* samples survived this atmospheric entry (Westall and de la Torre-Noetzel, 2008). It has been argued that either the 2 cm rock coverage was not thick enough to protect the microorganisms, or that hot gases released during ablation

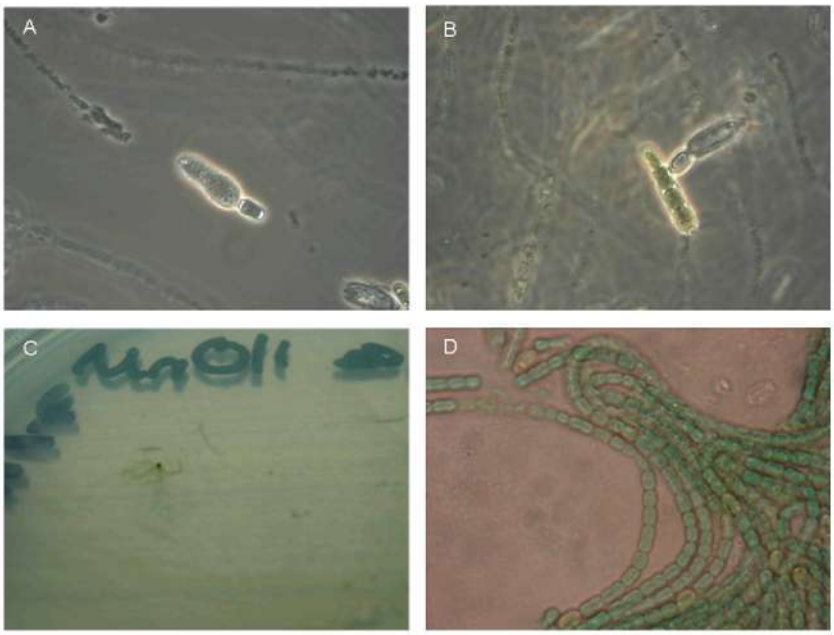

Fig. 5. The akinete preparation examined with 1000 times magnification (A); akinetes germinating into vegetative cells (B); *Anabaena cylindrica* growing out of a rock exposed to space conditions without UV exposure (C); chains of *Anabaena cylindrica* observed with 1000 times magnification (D).

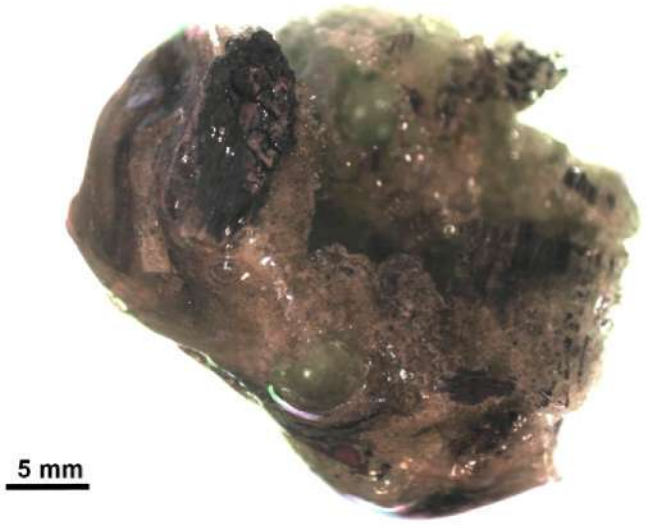

Fig. 6. The atmospheric re-entry process caused the general mineralogical and geochemical transformation of the granite into a glassy, nearly homogeneous material. Nevertheless, the detailed study of some centimetre-size fragments showed that some textural microdomains (e.g. smooth and rough surfaces, blebs, friction-striae-like features) can be distinguished.

pervaded the space between the sample and the sample holder leading to intense local heating. This assumption was confirmed by surface melting observed at the non-exposed surface of the rock samples (Brandstätter et al., 2008).

4. Discussion

The data from the *Lithopanspermia*/*Biopan* experiment clearly demonstrates the extraordinary survival capacity of lichens in outer space. Besides the lichens *R. geographicum* and *X. elegans*, which were already studied in the experiment LICHENS on board of *Biopan*-5 (Sancho et al., 2007, 2009; de los Ríos et al., 2010), the vagrant lichen *A. fruticulosa* showed a similar high resistance to all space parameters with regard to their photosynthetic activity: cosmic radiation, space vacuum as well as the full spectrum of solar extraterrestrial electromagnetic radiation (Table 1). Further studies of the gas exchange rate of flown and space exposed *A. fruticulosa* thalli revealed normal values of respiration and photosynthesis (Sancho et al., 2009).

The viability of the dark flight samples determined by LIVE/DEAD staining and CLSM analysis was slightly reduced, by about 20% (Table 1), and even more in the Sun-exposed samples (up to about 40%). We hypothesise that the mycobionts are especially vulnerable to space and that the high fraction of mycobionts in *R. geographicum*, might be the main reason for the measured reduction in viability. It seems that the photobionts in their natural symbiotic system were extremely well protected against the harsh environment of space. Protection was provided by the differentiated cortex with Sun-screening pigments as well as by live or dead cells of the surrounding fungus.

From the point of view of Lithopanspermia, the high resistance of ascospores is of particular importance, as they are protected by the fruiting bodies and by secondary lichen metabolites, such as parietin, carotene, emodin in *X. elegans*, (Edwards et al., 2003; Wynn-Williams and Edwards, 2002; Wynn-Williams et al., 2002) and melanin and usnic acid deposits in *R. geographicum* (BeGora and Fahselt, 2000). An additional protection is exerted by the presence of extrapolymeric substances (Kappen, 1988; Honegger, 1993; de los Ríos et al., 2002, 2003; Flemming and Wingender, 2001). In addition, desiccation caused by space vacuum, facilitated the germination capacity of the ascospores of *R. geographicum*. Ascospores in the fruiting bodies and photobiont cells from deep layers of the lichen thallus are the best protected cells and candidates for surviving natural transfer between planets (Mileikowsky et al., 2000). Together they comprise all prerequisites for generating a new lichen thallus on the new planet, if conditions are favourable for life.

In contrast to the high resistance of lichens, which are composed of cyanobacteria as photobiont and a fungus as mycobiont, cyanobacterial communities within their natural habitat, such as halite or limestone/sandstone, were less resistant to prolonged periods in outer space. It seems that vegetative cells of many naturally occurring cyanobacteria would lose viability once extracted from their natural environment. The limestone/sandstone rocks were predominantly colonised by *Leptolyngbya* species; however, 16S rDNA analysis and isolation experiments with freshly isolated rocks, demonstrated that the cyanobacteria community of the rocks were highly diverse (Olsson-Francis et al., 2010). In addition, in the vegetative state, cyanobacteria that survive isolation from their natural environment would be killed by the extreme conditions of space. However, some extremophilic cyanobacteria, such as an unidentified species of *Synechococcus* isolated from gypsum–halite crystals were reported to largely survive the harsh conditions of outer space experienced on a two-week flight on *Biopan*-1 (Mancinelli et al., 1998).

The experiments reported here suggest that many widespread cyanobacterial communities are not capable of surviving prolonged periods in outer space. However, resting state cyanobacteria are likely to confer greater resistance to space conditions. Akinetes are resting state cyanobacteria and are known to survive decades of desiccating conditions (Olsson-Francis et al., 2009). Furthermore, resting state cyanobacteria do not require sunlight and are therefore not limited to the upper surface of the rock. Thus, akinetes, or similar resting state phototrophic organisms, might provide a mechanism for escaping the adverse conditions experienced at the surface of the rock during atmospheric entry (Cockell et al., 2007; Cockell, 2008). These characteristics make resting cells of cyanobacteria one ideal model system for understanding the interplanetary transfer of microorganisms.

The space experiments reported here have shown that lichens can survive short-term, i.e. 10 days, exposure to outer space. From laboratory experiments mimicking impact ejection at shock pressures up to 50 GPa it is known, that photobiont cells and particularly ascospores of *X. elegans* are able to survive impact ejection (Stöffler et al., 2007; Horneck et al., 2008). The crucial stage in lithopanspermia seems to be the process of capturing by another planet and entry and landing on that planet. So far, in the *Lithopanspermia*/*Stone* experiment, all cells were killed during the entry process. In order to perform a more realistic simulation of the entry of meteorites carrying endolithic microorganisms, rock of different composition, e.g. basalts, should be tested and the technical concept of *Stone* needs to be modified so that larger, and in particular thicker rock samples can be accommodated. However, samples thicker than 2 cm can for safety reasons not be installed on a *Foton* capsule. The very thick and resistant heat shield of the *Foton* spacecraft will safely do its job even if equipped with recesses to accommodate *Stone* as it is now, but a further deepening of the recesses in the heat shield to make room for thicker *Stone* samples has justifiably been rejected by the *Foton* engineers and safety people.

Natural activities, such as rearrangements and relocations of soil material by glaciers or liquid water may lead to situations, where lichens, lichen fragments or its symbionts are better protected by soil material, and thereby better protected for all three steps of lithopanspermia. Therefore, on one hand, we cannot completely exclude the likelihood of a successful transfer of lichen material between two planets in the Solar System, on the other

hand, we are also not sure about the opposite (complete disintegration of biological material and no survival during interplanetary transfer of lichens). Further experiments in space and in simulation facilities on ground with different types of rock-colonising biological communities and rock material are envisaged to amend knowledge for assessing the likelihood of lithopanspermia.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Education and Science through CSIC (CGL2006-04658/BOS to A. de los Ríos, CGL2007-62875/BOS to J. Wierzchos), UCM (CGL2006-12179 to L. G. Sancho) and INTA (ESP2005-25292 to R. de la Torre), as well as by a grant of the Bundesministerium fuer Wirtschaft und Technologie through DLR (BMWi, 50WB0614 to S. Ott and J.P. de Vera) and the Heinrich-Hertz-Stiftung NRW. We are also grateful for assistance and logistic support during collection and habitat characterisation of the samples in the Alps to Ruth and Peter Inderbinen/Welschen (Walliserhof Zermatt) as well as to the pilots of AirZermatt and for technical help for sample preparation to Eva Posthoff (University of Düsseldorf) and Prof. Dr. Martin Melles and his team from the Institute of Geology and Mineralogy of the University Cologne. The authors thank Fernando Pinto, Sara Paniagua, Cesar Morcillo, Teresa Carnota and Gilberto Herrero for technical assistance and Ana Burton for help in translating some of the text. We thank Prof. Dr. Carmen Ascaso for her great scientific help in the development of this work.

Appendix A

A.1. Lithopanspermia flight hardware

A.1.1. Lithopanspermia on Biopan

One unit of the *Lithopanspermia* experiments was part of the payload of the *Biopan*-6 space mission of ESA. *Biopan* is a cylindrical exposure container of 38 cm in diameter for biological samples, installed on the outside of the re-entry capsule of a Russian *Foton* satellite (Fig. A1) (Demets et al., 2005; Baglioni et al., 2007). After reaching an orbital altitude of approximately 300 km, the lid of *Biopan* folds open thereby exposing the experimental samples to the outer space environment. *Biopan* is equipped with in-built sensors of temperature, pressure, and UV and VIS for monitoring space environmental parameters relevant for the experimental objectives (Horneck et al., 2001).

The hardware of *Lithopanspermia*/*Biopan* was constructed of aluminum–silica alloy-ISO Al Mg Si (Al6082 T6) and consisted of a top (level-1) and a bottom (level-2) plate, each dimensioned 146 mm × 129 mm × 23 mm, and each accommodating 36 cylindrical sample cells of 13 mm in diameter and 9 mm in height (Fig. A2). The cells of the top plate were covered by optical long-pass filters with the following characteristics: (i) MgF_2, which is transparent for the complete spectrum of extraterrestrial solar electromagnetic radiation of $\lambda > 110$ nm; (ii) SQ0 synthetic quartz transmitting solar electromagnetic radiation of $\lambda > 200$ nm, thereby simulating the UV and VIS radiation climate on the surface of Mars; (iii) long-pass filter for $\lambda > 290$ nm to simulate the terrestrial UV and VIS radiation climate (as a control) and (iv) for $\lambda > 400$ nm thereby cutting off all solar UV radiation. Reference samples in the bottom plate were kept in the dark during the whole mission. For allowing access of space vacuum to the samples a channel system

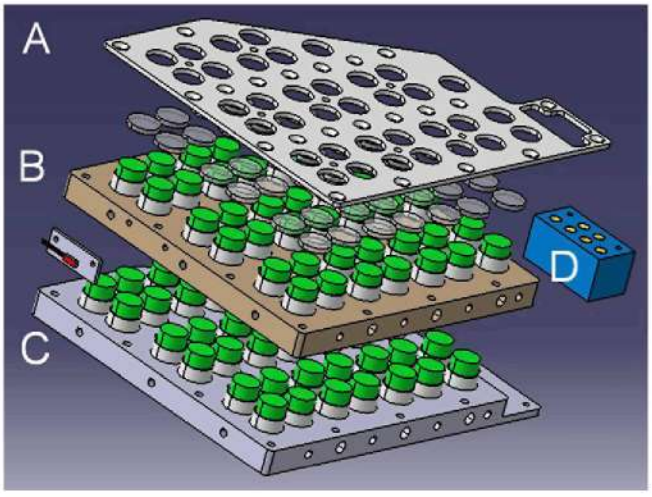

Fig. A2. Breackdown of *Lithopanspermia* elements; Top plate (A); level-1 (B); level-2 (C); *Litho-Dose* (D).

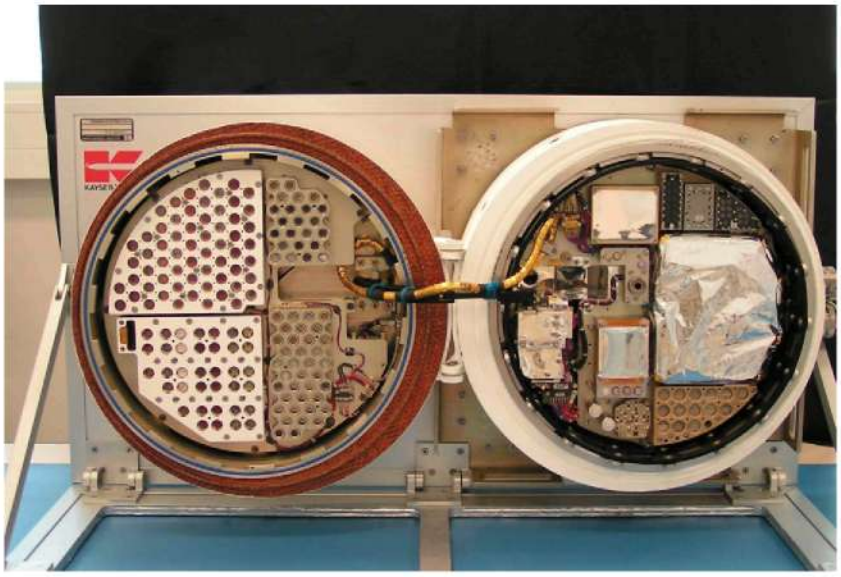

Fig. A1. *Biopan*-6, lid open, with experiment *Lithopanspermia* accommodated in the lid (lower left) showing the 36 sample cells of level-1 with cover plate, temperature sensor AD590 and termoluminiscence detector *Litho-Dose*.

was drilled in- and between the cells. A tubular membrane (polyethersulfon PES (371WPET12, Berghof Filtrations- und Anlagetechnik GmbH&Co KG, Eningen, Germany) with a pore size of 100 kD and a diameter of 12 mm was inserted in each cell to prevent possible contamination between adjacent cells through the channels. The samples inside the cells, and the optical filters on top of the cells, were fixed by a mixture of silicone and primer (RTV-576 with primer SS41555).

The hardware (except the optical filters) was covered by a white coated (paint SG121FD) thin plate (146 mm × 129 mm × 2 mm) to reduce temperature excursions and to limit the temperature gradient in relation with the *Biopan* structure. Mechanical function of this white plate was to clamp the optical filters and the experimental package of *Lithopanspermia* to *Biopan*'s mounting plate. Steel bolts (14 M5 bolts) were used to secure *Lithopanspermia* onto the lid plate of *Biopan* and to hide the bolts, which fixed the top and bottom plates (6 M5 bolts). Total mass of the experimental package was 636.7 g.

In addition to the *Biopan*-provided sensors, a thermoluminiscence dosimeter (TLD) assembly *Litho-Dose* (Fig. A2D) was integrated adjacent to the sample cells, to register the depth dose distribution of cosmic radiation (Reitz et al., 2002; Olko et al., 2006). On the opposite side, one of the AD590 temperature sensors from *Biopan* was located. The time profile of the radiation dose during the mission was recorded by another experiment on *Biopan*-6, called *R3D* (Dachev, 2008).

A.1.2. Lithopanspermia as part of Stone

The *Stone* experiments of ESA consisted of four disc-shaped test samples in the outer surface of the heat shield of the *Foton* M3 reentry capsule around the point where the spacecraft is subjected to the highest stress upon atmospheric entry (stagnation point). One of the test samples of *Stone* was the *Lithopanspermia* unit. It was composed of three parts: (i) the lichen *R. geographicum* on its natural granitic habitat (a disc of 45 mm diameter and 4 mm thickness); (ii) a spacer (a 2 mm thick ring made out of optical fibre G10FR4 with 70 mm outer and 40 mm inner diameter), which separated the sample from the holder at the surface of the capsule (Fig. A3A); and (iii) a cover of hat-like shape (with a base of 70 mm outer and 45 mm inner diameter and a 10 mm high tube with 45 mm inner diameter that was overarched by a dome of 6 mm height at its highest point), which protected the sample against the extremely high friction and temperature conditions reached during re-entry (Fig. A3B and C). This cover was designed to simulate the external layer of a meteorite. It was fabricated from the same material that was used for the ablative heat shield of the *Foton* capsule, i.e., glass textolite (a glass–fibre reinforced phenolic resin material). (Fig. A3B and C). The lichen-on-granite sample was accommodated between the spacer and the cover with the biological layer oriented inwards, towards the capsule. The three parts – spacer, lichen–granite sample and cover – were glued together as one block, using silicone RTV-566 with primer SS41555, and inserted in an annular *Stone* holder which was bolted into a recess in the skin of the *Foton* capsule, with the 6-mm high dome (Fig. A3B) protruding from the smooth *Foton* surface.

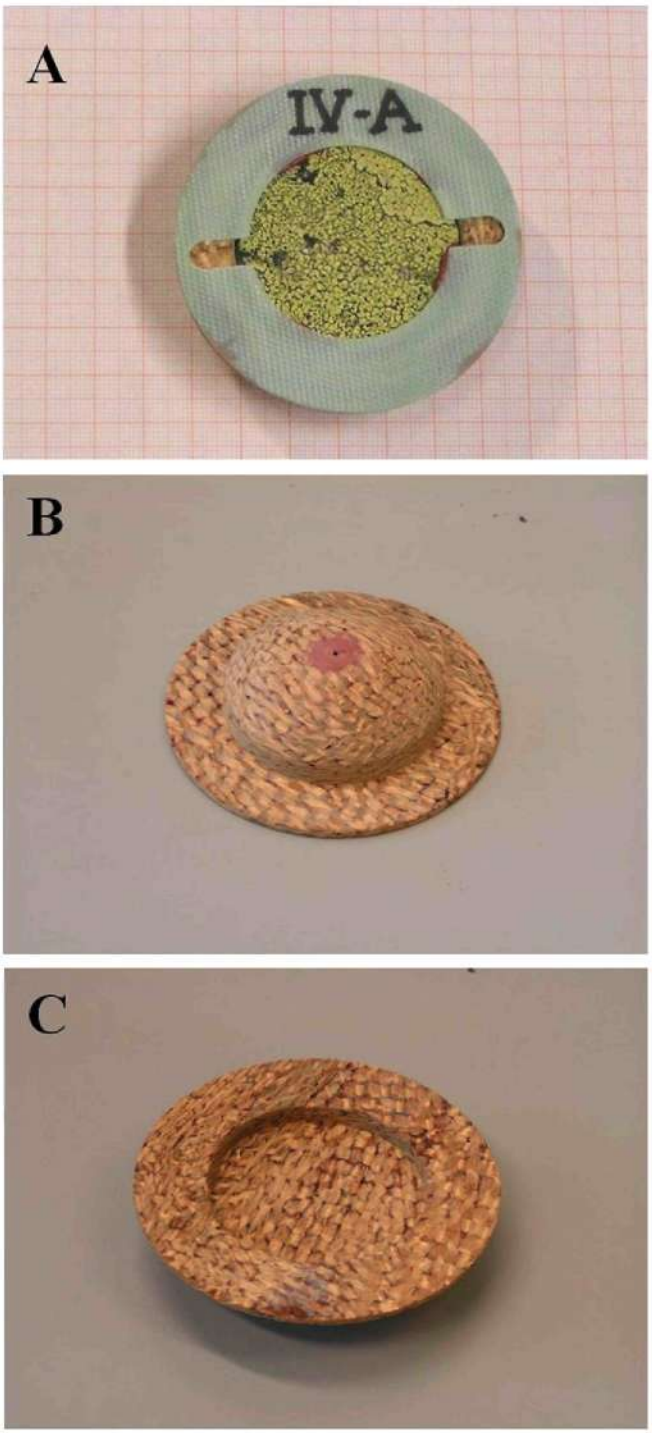

Fig. A3. Hardware of the *Lithopanspermia* unit on *Stone*: biological sample and spacer (A), external part of protection cup (B), inner part of protection cup (C).

A.2. Flight protocol

The *Lithopanspermia*/*Stone* sample (Fig. A3) was delivered to ESA's technical Center ESTEC in Noordwijk, The Netherlands, by the end of January 2007. It was then sent to Russia in February 2007 for integration in the skin of the *Foton* M3 capsule. For the *Lithopanspermia*/*Biopan* experiment, on the 12th of August 2007, at the principal investigators laboratory INTA, the biological samples were integrated in the different sample cells of the *Lithopanspermia* hardware according to the sample-test parameter plan. The experiment was then transported to ESTEC, where on 6th of September 2007 it was fixed in the lid of *Biopan*-6 (Fig. A1). The fully integrated *Biopan*-6 facility was then transported to the launch site Baikonur in Kazakhstan for accommodation on the *Foton* M3 satellite.

Foton M3 with *Biopan*-6 and *Stone* was launched on 14th of September 2007. It reached a near-circular orbit with a maximum apogee of 302 km and minimum perigee of 263 km. The orbital inclination was 63°. The orbital period of *Foton* M3 was 89.9 min, alternating Sun-illuminated periods with darkness periods,

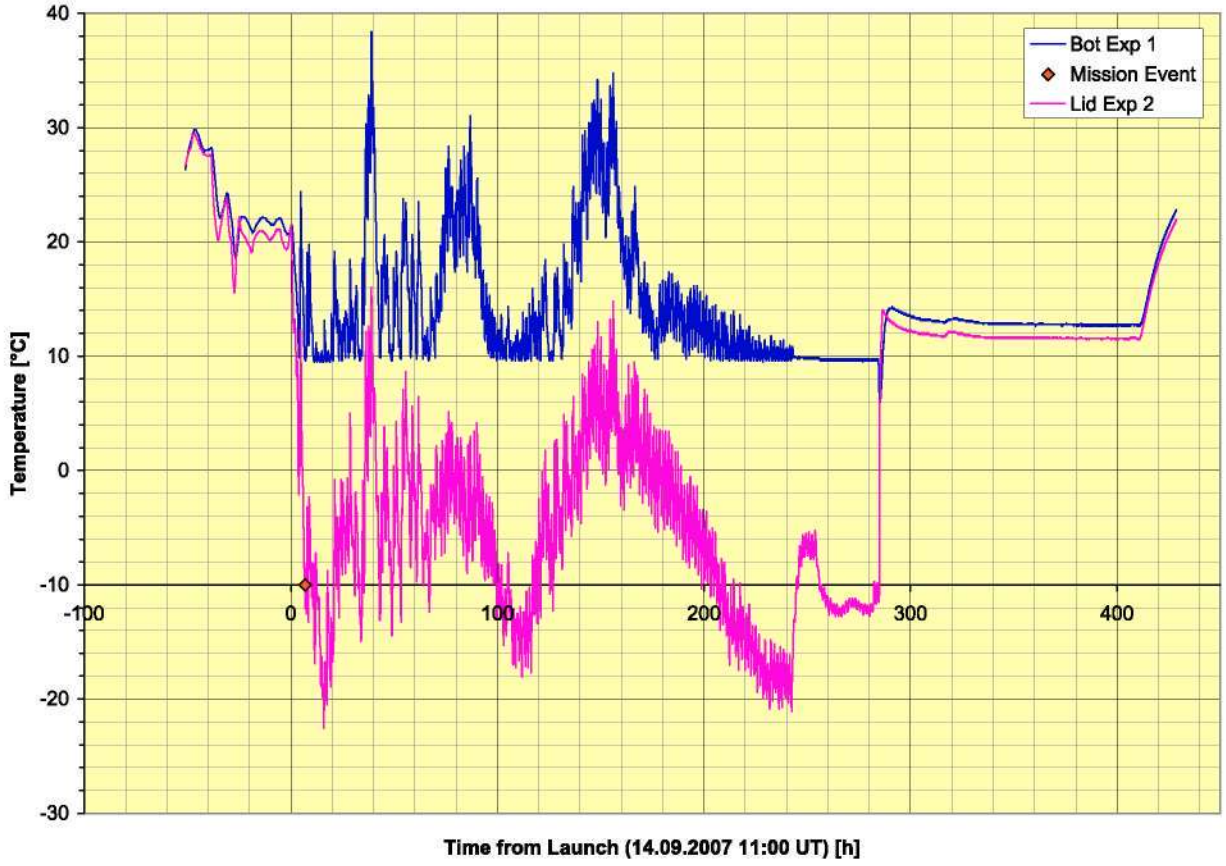

Fig. A4. Temperature profile of the *Biopan*-6 mission, measured in the bottom part (upper curve) and the lid (lower curve) of the facility.

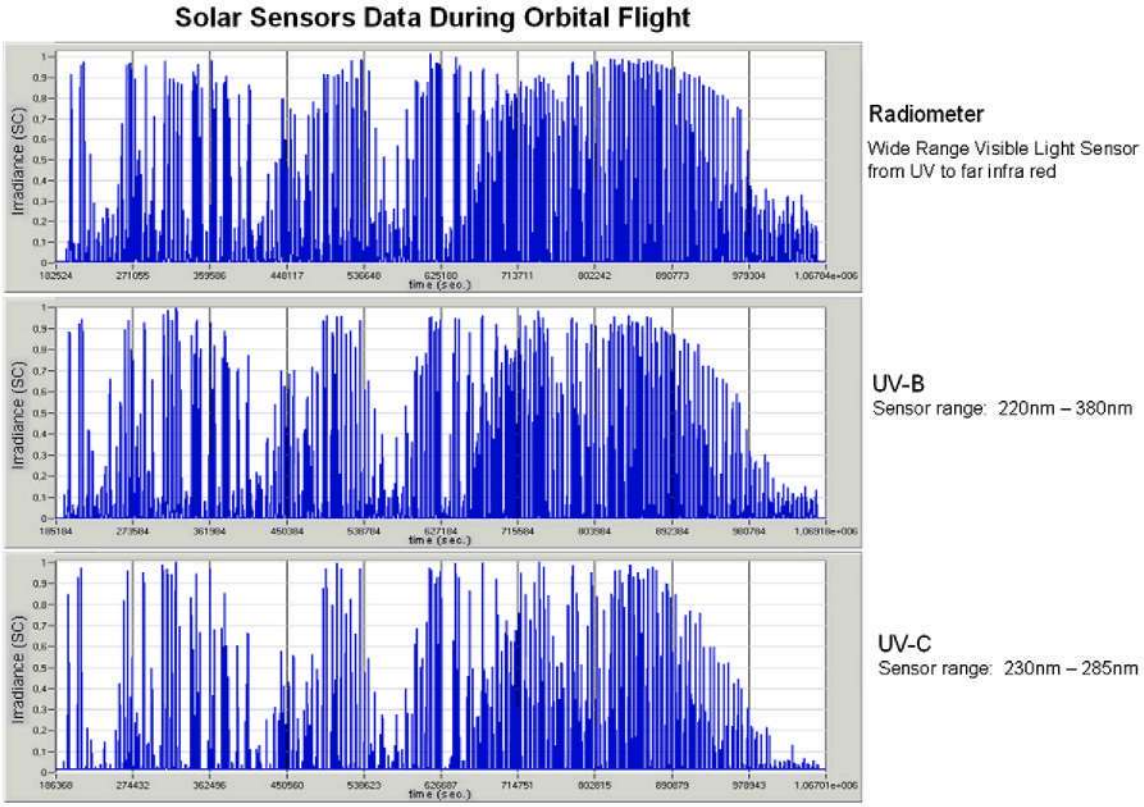

Fig. A5. Solar irradiance data of Lithopanspermia measured with solar sensors of different spectral sensitivity (courtesy of Kayser-Threde, München).

corresponding to the spacecraft crossing the night side of the Earth. Having completed 190 orbital loops in 12 days with the lid of *Biopan*-6 open for 10 days, the spacecraft landed in the vicinity of Kostanay, Kazakhstan on 26 September 2007. *Biopan*-6 and *Stone* were dismounted from the capsule and transported to ESTEC for de-integration of the samples and further analysis in the investigators' laboratory.

A.3. Lithopanspermia flight data

During each orbital loop, the *Foton* M3 satellite was about 35 min in the Earth's shadow and 55 min in the Sun, resulting in temperature fluctuations of about 10 °C per orbit. Temperature was further influenced by orbital parameters resulting in temperature fluctuations at the position of the *Lithopanspermia* hardware between −23 °C and +16 °C (Fig. A4). Because *Foton* is a non-stabilized free-flying satellite, which slowly rotates during its orbital journey, the samples were arbitrarily insolated for short intervals (minutes) depending on the orientation of the satellite (Fig. A5). The depth dose profile measured in the *Litho-Dose* experiment steeply declined with shielding mass from 100 ± 20 mGy at level-1 (top layer, Sun exposed) of the experiment down to 4 ± 0.5 mGy at level-2 (bottom layer, dark control samples) (Fig. A6). These data are comparable with those of previous *Biopan* missions (Table A1).

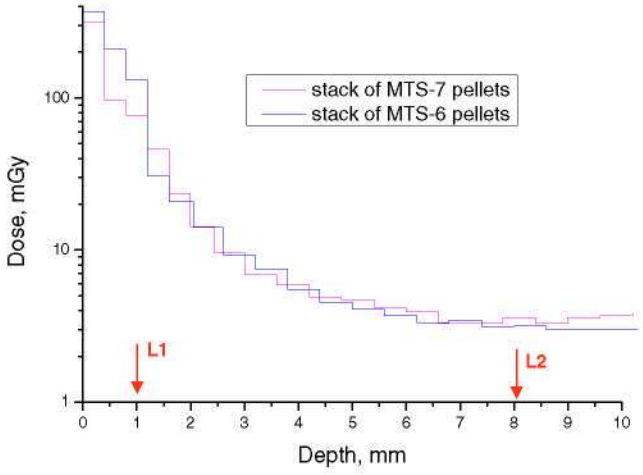

Fig. A6. Depth dose profile of cosmic radiation, measured in the *Litho-Dose* experiment. L1 is the position of the upper sample layer, L2 the position of the bottom dark sample layer.

Table A1
Environmental data from *Biopan*-1 through *Biopan*-6 missions (data for *Biopan*-1–3 from Horneck et al. (2001), for *Biopan*-5 from Sancho et al. (2007)).

BIOPAN no.	Flight date day/month/year	Exposure period (lid open) (h)	Solar UV > 170 nm (kJ m^{-2})	Cosmic radiation (mGy)	Temperature (°C)
1	14/06-02/07/1994	355	17 317	74.0[a]	−20 to +12
				5.9[b]	
2	09/10-23/10/1997	239	12 030	29.9[a]	−38 to +10
				4.0[b]	
3	09/09-24/09/1999	302	11 501	28.2 ± 0.6[a]	−17 to +15
				4.5 ± 0.1[b]	
5	31/05-15/05/2005	351	22 473	3.16[b]	−21.7 to +21.8
6	14/09-26/09/2007	240	11 800	100 ± 20[a]	−23.0 to +27.0
				4 ± 0.5[b]	

[a] Upper Sun-exposed layer of experiments in *Biopan* lid.
[b] Bottom dark reference layer of experiments in *Biopan* lid.

References

Arrhenius, S., 1903. Die Verbreitung des Lebens im Weltenraum. Die Umschau 7, 481–485.

Artemieva, N.A., Ivanov, B.A., 2004. Launch of martian meteorites in oblique impacts. Icarus 171, 183–196.

Baglioni, P., Sabbatini, M., Horneck, G., 2007. Astrobiology experiments in Low Earth Orbit: Facilities, instrumentation, and results. In: Horneck, G., Rettberg, P. (Eds.), Complete Course in Astrobiology. Wiley-VCH, Berlin, NY, pp. 273–320.

Becker, R.H., Pepin, R.O., 1984. The case for a martian origin of the shergottites: Nitrogen and noble gases in EETA 79001. Earth Planet. Sci. Lett. 69, 225–242.

BeGora, M.D., Fahselt, D., 2000. An alternative method for the quantification of lichen secondary products. The Bryologist 103 (3), 563–567.

Benardini, J.N., Sawyer, J., Venkateswaran, K., Nicholson, W.L., 2003. Spore UV and acceleration resistance of endolithic *Bacillus pumilus* and *Bacillus subtilis* isolates obtained from Sonoran desert basalt: Implications for lithopanspermia. Astrobiology 3, 709–717.

Berney, M., Hammes, F., Bosshard, F., Weilenmann, H.-U., Egli, T., 2007. Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry. Appl. Environ. Microbiol. 73, 3283–3290.

Brack, A., and 12 colleagues, 2002. Do meteorids of sedimentary origin survive terrestrial atmospheric entry? The ESA artificial meteorite experiment STONE. Planet. Space. Sci. 50, 763–772.

Brandstätter, F., and 10 colleagues, 2008. Mineralogical alteration of artificial meteorites during atmospheric entry: The STONE-5 experiment. Planet. Space Sci. 56, 976–984.

Büdel, B., Darienko, T., Deutschwitz, K., Dojani, S., Friedl, T., Mohr, K.L., Salisch, M., Reisser, W., Weber, B., 2009. Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microb. Ecol. 57, 229–247.

Clark, B.C., 2001. Planetary interchange of bioactive materials: Probability factors and implications. Orig. Life Evol. Biosph. 31, 185–197.

Cockell, C.S., 2008. The interplanetary exchange of photosynthesis. Orig. Life Evol. Biosph. 38, 87–104.

Cockell, C.S., Raven, J.A., 2004. Zones of photosynthetic potential on Mars and the early Earth. Icarus 169, 300–310.

Cockell, C.S., and 14 colleagues, 2007. Interplanetary transfer of photosynthesis: An experimental demonstration of a selective dispersal filter in planetary island biogeography. Astrobiology 7, 1–9.

Dachev, Ts.P., 2008. Analysis of the satellite radiation environment by the deposited energy spectrum. In: Proceedings of the Fundamental Space Research Conference, pp. 151–154, ISSBN: 978-954-322-316-9.

de la Torre, R., Garcia-Sancho, L., Horneck, G., 2007a. Adaptation of the lichen *Rhizocarpon geographicum* to harsh high-altitude conditions: Relevance to a habitable Mars. In: Cockell, C.S., Horneck, G. (Scientific eds.), ROME: Response of Organisms to the Martian Environment, ESA SP. 1299, European Space Agency, ESTEC, Noordwijk, The Netherlands, pp. 145–150.

de la Torre Noetzel, R., Sancho, L.G., Pintado, A., Rettberg, P., Rabbow, E., Panitz, C., Deutschmann, U., Reina, M., Horneck, G., 2007b. BIOPAN experiment LICHENS on the Foton M2 mission: Pre-flight verification tests of the *Rhizocarpon geographicum*-granite ecosystem. Adv. Space Res. 40, 1665–1671.

de los Ríos, A., Wierzchos, J., Sancho, L.G., Grube, M., y Ascaso, C., 2002. Microbial endolithic biofilms: A means of surviving the harsh conditions of the Antarctic. Proceedings of the Second European Workshop on Exo-/Astro-Biology.

de los Ríos, A., Wierzchos, J., Sancho, L.G., Ascaso, C., 2003. Acid microenvironments in microbial biofilms of Antarctic endolithic microecosystems. Environ. Microbiol. 54, 231–237.

de los Ríos, A., Wierzchos, J., Sancho, L.G., Green, A., Ascaso, C., 2005. Ecology of endolithic lichens colonizing granite in continental Antarctica. Lichenologist 37, 383–395.

de los Ríos, A., Ascaso, C., Wierzchos, J., Sancho, L.G., 2010. Space flight effects on lichen ultrastructure and physiology. Following the LICHENS 2005 experiment on board the BIOPAN V space exposure facility. In: Symbiosis and Stress. Series: Cellular Origins, Life in Extreme Habitats and Astrobiology (COLE). Springer, in press.

de Vera, J.-P., 2005. Grenzen des Überlebens: Flechten als Modellsystem für das Potential von Adaptationsmechanismen eines Symbioseorganismus unter Extrembedingungen. Inaugural-dissertation at the Heinrich-Heine-University, ULB Düsseldorf, pp. 1–180.

de Vera, J.-P., Horneck, G., Rettberg, P., Ott, S., 2003. The potential of lichen symbiosis to cope with extreme conditions of outer space – I. Influence of UV radiation and space vacuum on the vitality of lichen symbiosis and germination capacity. Int. J. Astrobiol. 1, 285–293.

de Vera, J.-P., Horneck, G., Rettberg, P., Ott, S., 2004a. The potential of the lichen symbiosis to cope with the extreme conditions of outer space. II: Germination capacity of lichen ascospores in response to simulated space conditions. Adv. Space Res. 33, 1236–1243.

de Vera, J.-P., Horneck, G., Rettberg, P., Ott, S., 2004b. In the context of Panspermia: May lichens serve as shuttles for their bionts in Space? Proceedings of the III European Workshop on Exo-Astrobiology. Mars: The Search for Life, Madrid,

Spain, 18–20 November 2003, ESA SP-545. European Space Agency, ESTEC, Noordwijk, The Netherlands, pp. 197–198.
de Vera, J.-P., Rettberg, P., Ott, S., 2008. Life at the limits: Capacities of isolated and cultured lichen symbionts to resist extreme environmental stresses. Orig. Life Evol. Biosph. 38, 457–468.
Demets, R., Schulte, W., Baglioni, P., 2005. The past, present and future of Biopan. Adv. Space Res. 36, 311–316.
Edwards, H.G.M., Newton, E.M., Wynn-Williams, D.D., Coombes, S.R., 2003. Molecular spectroscopic studies of lichen substances 1: Parietin and emodin. J. Mol. Struct. 648, 49–59.
Falcioni, T., Papa, S., Gasol, J.M., 2008. Evaluating the flow-cytometric nucleic acid double-staining protocol in realistic situations of planktonic bacterial death. Appl. Environ. Microb. 74, 1767–1779.
Fendrihan, S., Bérces, A., Lammer, H., Musso, M., Rontó, G., Polacsek, T.K., Holzinger, A., Kolb, C., Stan-Lotter, H., 2009. Investigating the effects of simulated martian ultraviolet radiation on *Halococcus dombrowskii* and other extremely halophilic Archaebacteria. Astrobiology 9, 104–112.
Flemming, H.C., Wingender, J., 2001. Biofilme – die bevorzugte Lebensform der Bakterien. Biologie in unserer Zeit 3, 169–180.
Friedmann, E.I., 1980. Endolithic microbial life in hot and cold deserts. Orig. Life 10, 223–235.
Fritz, J., Artemieva, N.A., Greshake, A., 2005. Ejection of martian meteorites. Meteorit. Planet. Sci. 9/10, 1393–1412.
Gauslaa, Y., Solhaug, K.A., 2004. Photoinhibition in lichens depends on cortical characteristics and hydration. Lichenologist 36, 133–143.
Gauslaa, Y., Ustvedt, E.M., 2003. Is parietin a UV-B or a blue-light screening pigment in the lichen *Xanthoria parietina*? Photochem. Photobiol. Sci. 2, 424–432.
Genge, M.J., Grady, M.M., 1998. The fusion crust of the stony meteorites: Implications for cosmic and fusion ablation spherules. <http://www.lpi.usra.edu/meetings/LPSC98/pdf/1808.pdf>.
Gladman, B., 1997. Destination Earth: Martian meteorite delivery. Icarus 130, 228–246.
Haugland, R.P., 2002. Handbook of Fluorescent Probes and Research Chemicals. Molecular Probes, Eugene, OR, pp. 217–314.
Head, J.N., Melosh, H.J., Ivanov, B.A., 2002. Martian meteorite launch: High-speed ejecta from small craters. Science 298, 1752–1756.
Honegger, R., 1993. Developmental biology of lichens. Tansley Review No. 60, New Phytol. 125, 659–677.
Horneck, G., 1993. Responses of *Bacillus subtilis* spores to space environment: Results from experiments in space. Orig. Life Evol. Biosph. 23, 37–52.
Horneck, G., Bücker, H., Reitz, G., Requardt, H., Dose, K., Martens, K.D., Mennigmann, H.D., Weber, P., 1984. Microorganisms in the space environment. Science 225, 226–228.
Horneck, G., Bücker, H., Reitz, G., 1994. Long-term survival of bacterial spores in space. Adv. Space Res. 14 (10), 41–45.
Horneck, G., Rettberg, P., Reitz, G., Wehner, J., Eschweiler, U., Strauch, K., Panitz, C., Starke, V., Baumstark-Khan, C., 2001. Protection of bacterial spores in space, a contribution to the discussion on Panspermia. Orig. Life Evol. Biosph. 31, 527–547.
Horneck, G., and 10 colleagues, 2008. Microbial rock inhabitants survive hypervelocity impacts on Mars-like host planets: First phase of Lithopanspermia experimentally tested. Astrobiology 8, 17–44.
Hughes, K.A., Lawley, B., 2003. A novel Antarctic microbial endolithic community within gypsum crusts. Environ. Microbiol. 5, 555–565.
Jones, K.H., Senft, J.A., 1985. An improved method to determine cell viability by simultaneous staining with fluorescein diacetate–propidium iodide. J. Histochem. Cytochem. 33, 77–79.
Jönsson, K.I., Rabbow, E., Schill, R.O., Harms-Ringdahl, M., Rettberg, P., 2008. Tardigrades survive exposure to space in Low Earth Orbit. Curr. Biol. 18, pR729–pR731.
Kappen, L., 1988. Ecophysiological relationships in different climatic regions. In: Galun, M. (ed.), CRC Handbook of Lichenology, Boca Raton, pp. 37–100.
Lammer, H., and 17 colleagues, 2009. What makes a planet habitable? Astron. Astrophys. Rev., in press. doi:10.1007/s00159-009-0019-z.
Mancinelli, R.L., White, M.R., Rothschild, L.J., 1998. Biopan-survival. I: Exposure of the osmophiles *Synechococcus* sp. (Nageli) and *Haloarcula* sp. to the space environment. Adv. Space Res. 22, 327–334.
Martinez-Frias, J., Weigel, A., Marti, K., Boyd, T., Wilson, G.H., Jull, T., 1999. The Getafe rock: Fall, composition and cosmic ray records of an unusual ultrarefractory scoriaceous material. Rev. Metal. 35, 308–315.
McKay, C.P., Friedmann, E.I., 1985. The cryptoendolithic microbial environment in the Antarctic cold desert: Temperature variations in nature. Polar Biol. 4, 19–25.
Melosh, H.J., 1984. Impact ejection, spallation, and the origin of meteorites. Icarus 59, 234–260.
Mileikowsky, C., Cucinotta, F., Wilson, J.W., Gladman, B., Horneck, G., Lindegren, L., Melosh, J., Rickman, H., Valtonen, M., Zheng, J.Q., 2000. Natural transfer of viable microbes in space, part 1: From Mars to Earth and Earth to Mars. Icarus 145, 391–427.
Moeller, R., Horneck, G., Rabbow, E., Reitz, G., Meyer, C., Hornemann, U., Stöffler, D., 2008. Role of DNA protection and repair in resistance of *Bacillus subtilis* spores to ultrahigh shock pressures simulating hypervelocity impacts. Appl. Environ. Microbiol. 74, 6682–6689.
Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J., Setlow, P., 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microb. Mol. Biol. Rev. 64, 548–572.
Nubel, U., Garcia-Pichel, F., Muyzer, G., 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. 63, 3327–3332.
Nybakken, L., Solhaug, K.A., Bilger, W., Gauslaa, Y., 2004. The lichens *Xanthoria elegans* and Cetraria islandia maintain a high protection against UV-B radiatioan in Arctic habitats. Oecologia 140, 211–216.
Olko, P., Marczewska, B., Czopyk, L., Czermak, M.A., Klosowski, M., Waligorski, M.P.R., 2006. New 2-D dosimetric technique for radiotherapy based on planar thermoluminescent detectors. Radiat. Prot. Dosim. 118, 213–218.
Olsson-Francis, K., de la Torre, R., Towner, M.C., Cockell, C.S., 2009. Survival of akinetes (resting-state cells of cyanobacteria) in Low Earth Orbit and simulated extraterrestrial conditions. Orig. Life Evol. Biosph. 39, 565–579.
Olsson-Francis, K., de la Torre, R., Cockell, C.S., 2010. Isolation of novel extreme-tolerant from a rock-dwelling microbial community by using exposure to low earth orbit. Appl. Environ. Microbiol. 76–7, 2115–2121.
Onofri, S., Barreca, D., Agnoletti, A., Rabbow, E., Horneck, G., de Vera, J.-P.P., Selbmann, L., Zucconi, L., Hatton, J., 2008. Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Mars conditions. Stud. Mycol. 61, 99–109.
Pedersen, K., 2000. Exploration of deep intraterrestrial microbial life: Current perspectives 185, 9–16.
Reitz, G., Facius, R., Bilski, P., Olko, P., 2002. Investigation of radiation doses in open space using TLD detectors. Radiat. Prot. Dosim. 100, 533–536.
Rettberg, P., Eschweiler, U., Strauch, K., Reitz, G., Horneck, G., Wänke, H., Brack, A., Barbier, B., 2002. Survival of microorganisms in space protected by meteorite material: Results of the experiment EXOBIOLOGIE of the PERSEUS mission. Adv. Space Res. 30, 1539–1545.
Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1–61.
Sancho, L.G., Schroeter, B., Del-Prado, R., 2000. Ecophysiology and morphology of the globular erratic lichen *Aspicilia fruticulosa* (Evers.) Flag. from Central Spain. Bibl. Lichenol. 75, 137–147.
Sancho, L.G., Palacios, D., De Marcos, J., Valladares, F., 2001. Geomorphological significance of lichen colonization: Hoya del Cuchillar de las Navajas, Sierra de Gredos (Spain). Catena 43, 323–340.
Sancho, L.G., De la Torre, R., Horneck, G., Ascaso, C., de los Ríos, A., Pintado, A., Wierzchos, J., Schuster, M., 2007. Lichens survive in space: Results from 2005 LICHENS experiment. Astrobiology 7, 450–454.
Sancho, L.G., de la Torre, R., Pintado, A., 2009. Lichens, new and promising material from experiments in astrobiology. Fungal Biol. Rev., in press. doi:10.1016/fbr.2008.12.001.
Schreiber, U., Bilger, W., Neubauer, C., 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D., Cadwell, M.M. (Eds.), Ecophysiology of Photosynthesis, vol. 1. Springer, Berlin, Heidelberg, NY, pp. 49–70.
Solhaug, K.A., Gauslaa, Y., 2004. Photosynthates stimulate the UV-B induced fungal anthraquinone synthesis in the foliose lichen *Xanthoria parietina*. Plant, Cell Environ. 27, 167–176.
Solhaug, K.A., Gauslaa, Y., Nybakken, L., Bilger, W., 2003. UV-induction of Sun-screening pigments in lichens. New Phytol. 158, 91–100.
Stivaletta, N., Barbieri, R., 2009. Endoliths in terrestrial arid environments: Implications for Astrobiology. In: Seckbach, J. (Ed.), From Fossils to Astrobiology, Records of Life on Earth and Search for Extraterrestrial Biosignature, Cellular Origin, Life in Extreme Habitats and Astrobiology, vol. 12. Springer, Berlin, Heidelberg, NY, pp. 319–333.
Stöffler, D., Horneck, G., Ott, S., Hornemann, U., Cockell, C.S., Moeller, R., Meyer, C., de Vera, J.-P., Fritz, J., Artemieva, N.A., 2007. Experimental evidence for the potential impact ejection of viable microorganisms from Mars and Mars-like planets. Icarus 186, 585–588.
Thompson, W. (Lord Kelvin). 1871. Presidential address to the British Association. In Popular lectures and addresses. MacMillan and Company, England, pp. 132–205.
Vickery, A.M., Melosh, H.J., 1987. The large crater origin of SNC meteorites. Science 237, 738–743.
Villarino, A., Bouvet, O.M.M., Regnaulta, B., Martin-Delautrea, S., Grimont, P.A.D., 2000. Exploring the frontier between life and death in *Escherichia coli*: Evaluation of different viability markers in live and heat- or UV-killed cells. Res. Microbiol. 151, 755–768.
Warren, P., 1994. Lunar and martian meteorite delivery services. Icarus 111, 338–363.
Weiss, B.P., Kirschvink, J.L., Baudenbacher, F.J., Vali, H., Peters, N.T., Macdonald, F.A., Wikswo, J.P., 2000. A low temperature transfer of ALH84001 from Mars to Earth. Science 290, 791–795.
Westall, F., de la Torre-Noetzel, R., 2008. Meteorites: Stones with stowaways? In: Looking Up: Europe's Quiet Revolution in Microgravity Research. Scientific American Inc., New York, NY, pp. 8–15.
Wierzchos, J., Ascaso, C., McKay, C.P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology 6, 415–422.
Wynn-Williams, D.D., Edwards, H.G.M., 2002. Environmental UV radiation: Biological strategies for protection and avoidance. In: Horneck, G., Baumstark-Khan, C. (Eds.), Astrobiology. The Quest for the conditions of Life. Springer, Berlin, Heidelberg, NY, pp. 245–258.
Wynn-Williams, D.D., Edwards, H.G.M., Newton, E.M., Holder, J.M., 2002. Pigmentation as a survival strategy for ancient and modern photosynthetic microbes under high ultraviolet stress on planetary surfaces. Int. J. Astrobiol. 1, 39–49.
Yamamoto, Y., 1975. Effect of desiccation on the germination of akinetes of *Anabaena cylindrica*. Plant Cell Physiol. 16, 749–752.